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RAS's Cloak of Invincibility Slips at Last?

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KRAS is the most frequently activated oncogene in human cancer, but it has, so far, shrugged off all attempts to inhibit its function directly. However, a recent report provides an entirely new approach to blocking mutant KRAS with small molecules and has the added benefit of sparing the wild-type protein.

Closely related protein isoforms encoded by the *RAS* family of oncogenes, *KRAS, NRAS*, and *HRAS*, are the most frequently activated drivers of human cancer (Pylayeva-Gupta et al., 2011). With some variations across populations dependent on smoking rates and ethnicity, *RAS* activating mutations are found in about 20% of cancers, with *KRAS* being the most frequently mutated, then *NRAS*, common in hematological malignancies, and *HRAS* a distant third. RAS proteins are thus exceptionally important players in cancer and represent an extraordinarily well-validated drug target. However, very little progress has been made in developing drugs that directly inhibit RAS protein function. A recent report provides a major advance in this area and, for the first time, brings into view the possibility that effective RAS inhibitory drugs might be on the horizon (Ostrem et al., 2013).

RAS has long been referred to by the pharmaceutical industry as "undruggable," implying that its structure is such that no direct small molecule inhibitors can be developed using current technology. The pharmaceutical industry has remained wary of RAS, in part due to the costly failure of farnesyl-transferase inhibitors, developed in the 1990s to inhibit the posttranslational isoprenylation of RAS that is essential for its biological function. Good farnesyl-transferase inhibitors were rapidly obtained, but proved ineffective in the clinic due to both alternative processing enzymes and a lack of specificity for RAS. Targeting different aspects of RAS posttranslational processing has been proposed since then, most recently with the identification of prenyl-binding protein phosphodiesterase δ inhibitors (Zimmermann et al., 2013), but it remains hard to see where the specificity for RAS would be achieved, because more than 60 different human proteins have been found to be farnesylated.

RAS sits atop a bifurcating network of signaling pathways; in the last decade, most attention has focused on inhibiting RAS function through the targeting of kinases in the pathways that it controls. These include protein kinases RAF, MEK, ERK, and AKT, and the lipid kinase phosphatidylinositol 3-kinase (PI3K). Inhibitors of these enzymes are either clinically approved or in advanced clinical trials. However, their progress in the clinic has not been smooth. In particular, given RAS's ability to activate multiple pathways, it has been reasoned that combined targeting of pathways may be needed to effectively reverse RAS function, but such drug combinations have proved to be significantly toxic.

An alternative approach has been to try to develop drugs that will bind to RAS directly and inhibit its function. In theory, it might be possible to prevent RAS from binding to GTP, the nucleotide required for RAS to adopt an activated conformation capable of interacting with downstream effector enzymes such as RAF and PI3K. However, the extraordinarily high affinity of RAS for GTP makes this extremely difficult, essentially ruling out the approach taken successfully with the ATP binding site of many kinases. Instead, attention has focused on finding RAS-binding small molecules that will block RAS's interaction with downstream signaling proteins. High-throughput screening approaches have been applied to this problem over many years, for example, looking for disrupters of RAS-RAF interaction. This has proved frustrating, confirming industry suspicion of protein-protein interaction inhibitor approaches, especially when the protein interfaces involved are large and relatively featureless, as is the case for RAS and its downstream effectors. Although there have been some promising initial results (Kato-Stankiewicz et al., 2002), these have not led to the development of new drugs.

The search for RAS inhibitors has escalated recently with the application of structure-based approaches to the discovery of direct binding molecules. A cell permeable synthetic α helix has been developed that inhibits the interaction of RAS with its upstream activating guanine nucleotide exchange factor SOS (Patgiri et al., 2011). However, although this will prevent wild-type RAS activation by upstream regulators such as receptor tyrosine kinases, it would not be expected to inhibit oncogenic mutant RAS, which is locked in an activated GTP-bound form and hence independent of upstream signal input. In addition, two NMR-spectroscopy-based small drug fragment library screens have successfully yielded direct KRAS binding moieties (Maurer et al., 2012; Sun et al., 2012). Both studies identified the same binding pocket on KRAS adjacent to the GTP-dependent conformational switch I/II regions. Unfortunately, occupation of this pocket with the drug fragments blocks KRAS-SOS interaction, but not interactions with downstream enzymes such as RAF, meaning that, by themselves, they are unlikely to make good inhibitors of mutant KRAS in cancer. However, these findings could provide a starting point to design inhibitors that could reach around to nearby regions of KRAS that are involved in effector interaction. A third report on RAS interaction inhibitors took an in silico screening approach based on the structure of a close RAS relative, MRAS, to find compounds that would bind GTP-loaded HRAS and block its interaction with RAF (Shima et al., 2013).

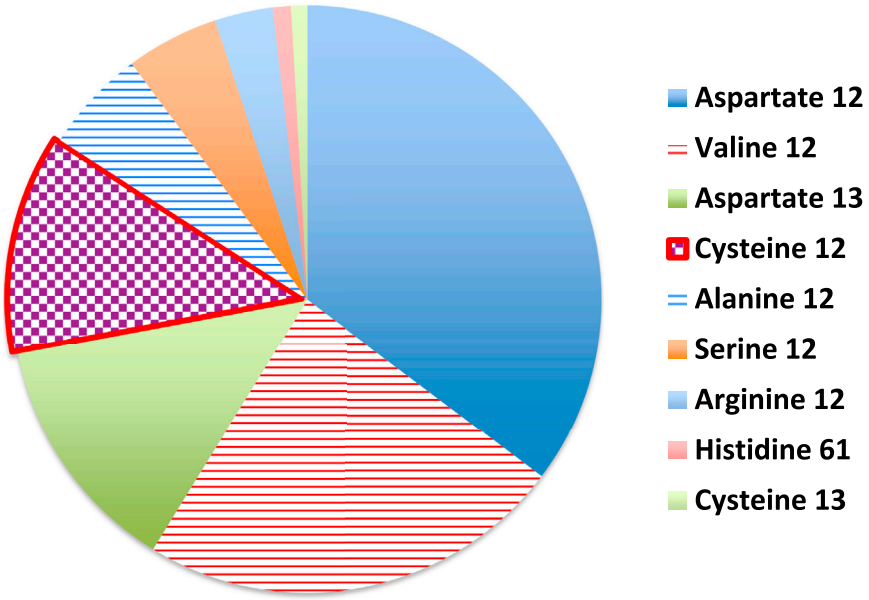

Figure 1. The Distribution of Mutations in KRAS Found in Human Cancers
Ostrem et al. (2013) have targeted the cysteine 12 mutant, outlined in red, which accounts for about 12% of total KRAS mutations and is found in about 2% of all human cancers. Common KRAS mutations involve substitution of reactive amino acid side chains, which may be targetable by similar approaches, with the exception of valine and alanine at codon 12, marked here by horizontal crosshatches. Data derived from the COSMIC database (http://cancer.sanger.ac.uk/cancergenome/projects/cosmic/).

These compounds bind RAS close to the junction of the switch I and switch II regions, but in a distinct orientation to those described above. However, with an ~20 μM IC_{50} for the lead compound, Kobe0065, these inhibitors remain far from being realistic drugs.

In the new work from Kevan Shokat's labaoratory, a quite distinct approach was taken to finding direct inhibitors of RAS proteins (Ostrem et al., 2013). They chose to target a common activated mutant form of KRAS, bearing a glycine-to-cysteine substitution at codon 12. This is the fourth most common KRAS mutation in human cancer, accounting for one in eight KRAS mutations overall (see Figure 1). As a tobacco smoke induced mutation, its occurrence is particularly high in lung cancer, where it is the most common KRAS mutation, occurring in 40% of KRAS mutant lung tumors. Overall, 8% of all lung cancers express this mutant KRAS protein, implicating it in over 100,000 lung cancer deaths a year worldwide.

The cysteine residue forms a chemically tractable anchor, which Ostrem et al. (2013) used to tether compounds, allowing exploration of interactions with nearby regions of the KRAS protein, including those involved in effector enzyme interactions. Initial compounds formed disulphide interactions with the cysteine, but this was subsequently changed to more stable reactive groups, still yielding covalent interactions and hence irreversible inhibitors. These inhibitors reduced the viability of KRAS-G12C-bearing lung cancer cells in culture, although it is clear that the inhibitors will require considerable further optimization before being suitable for use as drugs. A possible limitation to their effectiveness could lie in their apparent preference for the GDP-bound form of KRAS. Although helpful in locking mutant KRAS into an inactive state, the compound might not be able to prevent the initial GTP loading reaction as newly synthesized KRAS protein folds. Due to the slow hydrolysis of GTP on mutant KRAS, this could limit the degree to which KRAS activity can be suppressed.

Although the compounds described by Ostrem et al. (2013) are only an initial step, they do represent a remarkable advance in the struggle to target RAS proteins directly. The selectivity for a common activating mutant form of the protein could be advantageous over compounds that equally target wild-type protein and may have broader toxicities. Mutant-selective inhibitors for other oncogene products such as EGFR have recently entered clinical trials, and there are indications of significantly less toxicity than inhibitors that also target the normal protein (Walter et al., 2013). Although the G12C-specific inhibitors are unlikely to target other mutant versions of KRAS protein, most mutations found in cancer involve substitution of reactive amino acid side chains, which might be targetable by closely analogous methods. A particularly attractive mutant in this regard would be G12D, the most common KRAS mutation accounting for a third of all KRAS mutations in cancer (three times more prevalent than G12C). After decades languishing in the shadows of perceived undruggability, it seems that RAS is finally stepping into the spotlight as a tractable drug target in human cancer.

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